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Profiling and quantitative evaluation of three Nickel-Coated magnetic matrices for purification of recombinant proteins: lelpful hints for the optimized nanomagnetisable matrix preparation

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Abstract

Background: Several materials are available in the market that work on the principle of protein magnetic fishing by their histidine (His) tags. Little information is available on their performance and it is often quoted that greatly improved purification of histidine-tagged proteins from crude extracts could be achieved. While some commercial magnetic matrices could be used successfully for purification of several His-tagged proteins, there are some which have been proved to operate just for a few extent of His-tagged proteins. Here, we address quantitative evaluation of three commercially available Nickel nanomagnetic beads for purification of two His-tagged proteins expressed in Escherichia coli and present helpful hints for optimized purification of such proteins and preparation of nanomagnetisable matrices.

Results: Marked differences in the performance of nanomagnetic matrices, principally on the basis of their specific binding capacity, recovery profile, the amount of imidazole needed for protein elution and the extent of target protein loss and purity were obtained. Based on the aforesaid criteria, one of these materials featured the best purification results (SiMAG/N-NTA/Nickel) for both proteins at the concentration of 4 mg/ml, while the other two (SiMAC-Nickel and SiMAG/CS-NTA/Nickel) did not work well with respect to specific binding capacity and recovery profile.

Conclusions: Taken together, functionality of different types of nanomagnetic matrices vary considerably. This variability may not only be dependent upon the structure and surface chemistry of the matrix which in turn determine the affinity of interaction, but, is also influenced to a lesser extent by the physical properties of the protein itself. Although the results of the present study may not be fully applied for all nanomagnetic matrices, but provide a framework which could be used to profiling and quantitative evaluation of other magnetisable matrices and also provide helpful hints for those researchers facing same challenge.

Background

After introduction of metal chelate affinity chromatography, a new approach to protein fractionation [1] and describing a new chelating matrix, Ni-NTA, for purification of fusion proteins containing histidine tags [2,3], His-tag affinity purification has been widely used for the purification of recombinant proteins from various expression systems [4-6]. In recent years, a broad array of common support matrices with slightly different materials, magnetic properties, adsorbent particle size and shape, and spatially binding capacities and strengths have been introduced as tricky reagents for successful purification process of His-tagged proteins [7,8].

With respect to these properties, the matrices offered by different commercial vendors differ very substantially from one another. Indeed, the choice of matrix is complicated by the fact that various suppliers offer practically the same particles under different names [7]. A collection of suppliers for nanomagnetic beads

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intervals of induction process, centrifuged and the pellets were directly suspended in 150 μl of 5× loading buffer, shacked vigorously and then processed as above. Prestained protein ladder was used as molecular weight marker. Electrophoresis was performed in a Mini-Protean II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) with running buffer composed of 25 mM Tris-HCl pH 8.3, 192 mM glycine, 0.1% SDS. After separation, gels were stained with silver nitrate. Western blot analysis was carried out according to the protocol we published elsewhere [24] with some modifications. Briefly, after transfer onto nitrocellulose membranes, blocking was done overnight in 5% skimmed milk followed by three washes with TBS-TT (20 mM Tris base, 500 mM NaCl, 0.1% v/v Tween 20, 0.4% v/v Triton x100 PH, 7.5), each for 10 min. Goat anti-His6 monoclonal antibody (Invitrogen, California, USA) and rabbit anti-Mre11 and anti-ProT polyclonal antibodies (Produced in our laboratory) were applied to the membrane at 1:3000 as primary antibody for 1.5 h followed by 1:3000 dilution of hoarse-radish peroxidase (HRP)-conjugated rabbit anti-goat or sheep anti-rabbit (Avicenna Research Institute, Tehran, Iran) for 1 h. Membrane was then washed as above and specific bands were developed by enhanced chemiluminiscent (ECL) system (GH Healthcare, Buckinghamshire, UK) according to the manufacturer's instruction using X-ray film processor (HOPE Micro-Max, Warminster, USA).

Densitometric analysis

Silver-stained SDS-PAGE gels were scanned and density of specific bands for two recombinant proteins from samples collected at different bead concentration and each recombinant protein. The sum of the specific band densities from FT, W1-4, E1-4 and RF were set to 100%. Percent of band density in FT subtracted from 100% was defined as specific binding capacity. Purification yield was defined as the sum of the percents of the specific band densities at four elution steps (E1-4). Recovery percent was calculated as the percent of purification yield divided by specific binding capacity. The sum of the percents of specific band densities in W1-4 and RF was defined as protein loss.

Statistical Analysis

Numerical data analysis was done using SPSS software version 13.0 (SPSS Inc., Chicago, Illinois). Two-tailed statistical analyses were performed using the SPSS software version 13.0. Percent of bound, lost and eluted fractions of each protein was calculated for five individual experiments for each matrix and compared by Mann-Whitney test with Bonferroni correction. P-values less than 0.05 were considered significant.

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Authors' contributions

The authors meet the criteria for authorship as follows:
MRN has made substantial contribution to design, acquisition of data and manuscript drafting. MC has made substantial contribution to conception and design. SZ has participated in data analysis and AHZ has involved in methodology design, interpretation of data, critical revision of the manuscript and final approval of the version to be published.